REMARKS

Claims

Claims 29, 61, 63, 65, and 75-79 were considered in the non-final Office Action of December 30, 2009. Claims 34-60, 62, and 67-74 stand withdrawn from consideration. Claims 65 and 76 stand objected to and claims 29, 61, 63, 65, and 75-79 stand rejected.

Claims 29 and 61 are amended herein to clarify that the tissue sample is a skin tissue sample comprising dermis, epidermis, and a dermal-epidermal junction therebetween. Claims 29 and 61 have also been amended to provide that the cells are harvested from the dermis and the epidermis. These amendments are supported in the specification as filed at least at page 11, lines 9-11 and page 22, lines 10-14. Claims 29 and 61 have also been amended to recite that the composition has a cell population comprising keratinocyte basal cells, melanocytes and fibroblasts. This amendment is supported in the specification as filed at least at page 8, lines 18-21; and at page 12, lines 26-30. Further, claims 29 and 61 have been amended to recite that the composition and the tissue sample have cell compositions that are comparable. Claim 29 has also been amended to recite the "cell suspension" where appropriate to provide proper antecedent basis.

Claim 75, from which claims 78 and 79 indirectly depend, has been amended to clarify that the cells are harvested in a method using a solution that comprises an enzyme. This amendment is supported in the specification as filed at least at page 9, lines 16-22. In view of the amendment to claim 75, claims 78 and 79 have been amended to provide proper antecedent basis.

Further, as suggested by the Examiner, claim 65 has been amended to recite "comprises", and claim 76 is amended to recite "trypsin-EDTA" to correct for clerical errors.

No new matter has been added herein. Applicants have amended certain claims solely to expedite prosecution of the application. In making these amendments, Applicants are not acquiescing to the pending rejections and are not abandoning or surrendering any of the subject matter in previous versions or listings of the claims or in the application. Accordingly, Applicants reserve the right to pursue claims of similar, narrower, or broader scope in the future.

In view of the amendments to the claims and the following remarks, together with the affidavit under 37 C.F.R. § 1.132 by Dr. Fiona M. Wood (submitted herewith), Applicants respectfully request reconsideration and withdrawal of all claim objections and rejections.

Summary of Interview

As an initial matter, Applicants wish to thank the Examiner for the telephonic interview of March 23, 2010, during which the Examiner, the undersigned attorney, and Dr. William Dolphin were present. The presently-claimed invention and the following prior art references were discussed at the Interview: Noel-Hudson et al. (1993, *In Vitro Cell and Developmental Biology – Animal* 31: 508-515, reference C6 on 6/1/04 IDS, "Noel-Hudson"); and Hirobe (1991, *Journal of Experimental Zoology* 257: 184-194; reference U, "Hirobe 1991"). In discussing Noel-Hudson, Applicants noted that Noel-Hudson describes certain methods, in part, by reference to Boyce, S.T., Ham, R.G., "Cultivation, frozen storage and clonal growth of normal human epithelial keratinocytes in serum-free media." *J. Tissue Cult. Methods* 9:83-93 (1985) ("Boyce and Ham")[1], and Applicants further discussed Boyce and Ham. In addition, during the Interview, the rejections made under 35 USC 112 were discussed, and Applicants described certain amendments reflected herein. As required under MPEP § 713.04, Applicants respectfully supplement the Examiner Interview Summary mailed March 25, 2010 with the following additional information:

(A) No exhibit was shown and no demonstration was conducted during the Interview;

(B) Certain limitations present in independent claims 29 and 61 were discussed during the Interview;

(C) Noel-Hudson, Hirobe, and Boyce and Ham were discussed during the Interview;

(D) The amendments made herein were discussed by Applicants during the Interview;

(E) The general thrust of the principal argument of the Applicants was that the references fail to disclose the elements of the invention as claimed, including a composition of cells harvested from a tissue sample comprising dermis, epidermis and a dermal-epidermal junction therebetween; the composition being free of xenogenic serum and cellular congregates greater that 200 μM, and having a cell population comprising

[1] A copy of Boyce and Ham is submitted herewith in an Information Disclosure Statement.

keratinocyte basal cells, melanocytes and fibroblasts, wherein the cell population of the composition and the tissue sample are comparable.

(F) No other pertinent matters were discussed during the Interview; and

(G) The Interview did not result in any agreement between the Applicants and the Examiner.

Objection to Claims 65 and 76

Claims 65 and 76 were objected to for informalities. As discussed above, Applicants have amended claims 65 and 76 according to the Examiner's suggestion. Accordingly, Applicants respectfully request reconsideration and withdrawal of the objections to claims 65 and 76.

Rejection Under 35 U.S.C. § 112, Second Paragraph

In the Office action, claims 29, 61, 63, 65, and 75-79 were rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded by the Applicants as the invention. Specifically, the Examiner requested clarification of "dermal-epithelial" and "a ratio…comparable" in claims 29 and 61, "derived" in claim 61, and trypsin concentration in claims 78 and 79.

Regarding the recitation of "dermal-epithelial", Applicants have amended claims 29 and 61 to delete the reference to the dermal-epithelial junction and to recite that the tissue sample is a skin tissue sample comprising dermis, epidermis, and a dermal-epidermal junction therebetween. Claims 29 and 61 have also been amended to recite that the cell composition is produced by harvesting cells from both the dermis and epidermis, and that the cell composition thus produced comprises keratinocyte basal cells, melanocytes, and fibroblasts. As amended, Applicants respectfully submit that claims 29 and 61 are not indefinite with respect to the recitation of the sample or the location from which the cells are obtained.

Regarding the recitation of "a ratio…comparable", the Examiner stated that the use of the term "ratio" in connection with three value was confusing. In addition, the Examiner indicated that the criteria for determining whether one ratio is comparable to another are not clear. In response, Applicants have amended claims 29 and 61 to delete the reference to a ratio and to

recite that the cell population of the composition (which comprises keratinocyte basal cells, melanoctyes, and fibroblasts) and tissue sample are comparable—i.e., similar. With regard to "comparable," Applicants respectfully submit that this expression is clear and definite in the context of the claimed method of producing a cell suspension from a tissue sample. As provided in the specification at page 12, lines 23-30:

> "Another unique feature of the cell suspension produced according to the method of the first aspect of the invention is that the composition of cells in the cellular preparation is comparable to that seen in situ compared to prior art cellular preparation. One possible explanation for this is that in the prior art, culture of the cellular preparation utilises selective culture for keratinocytes, therefore loss of cellular constituents such as fibroblasts and melanocytes occurs whereas the cellular suspension produced from the first aspect of the invention has a cell composition comparable to the in situ cell population."

In addition, Applicants explain in the specification at page 8, lines 18-24 that:

> "It provides a means to produce a suspension of cells in a ratio to each other comparable with those seen in situ. That is, due to the manner of preparation of the cellular suspension, cells such as keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes typically have enhanced survival rates in comparison to standard tissue culture techniques, whereby selective cell culture can result in the loss of certain cell types."

As such, it would be clear to a person of ordinary skills in the art that Applicants' methods (e.g., obtaining cells from the both dermis and epidermis of a skin tissue sample; use of a nutrient solution as opposed to a selective culture medium; and no separating or filtering of any particular cell type or tissue type) would produce a composition having a population of cell types that is comparable—i.e., similar—to the cell population of the tissue sample from which the composition is produced.

In addition to the foregoing, Applicants respectfully submit herewith an affidavit under 37 C.F.R. § 1.132 by Dr. Fiona M. Wood, which further confirms that Applicants' "cell population of the composition and the tissue sample are comparable (i.e., similar)." *Id.* at paragraph 5A. Therefore, in view of the above clarification and the affidavit, Applicants respectfully submit that the "comparable" expression is clear and definite.

Claim 61 has also been amended to clarify that the cell suspension comprises cells "harvested" from both the dermis and the epidermis. Applicants believe that this amendment address the Examiner's question regarding "derived."

Claim 75, from which claims 78 and 79 indirectly depend, has been amended to clarify that the cells are harvested in a method using a solution that comprises an enzyme. Applicants believe that these amendments clarify that the recited concentration of trypsin relates to the solution used in the harvesting method of the cells, as the Examiner had suggested. As such, Applicants believe these amendments fully address the Examiner's clarity rejection to claims 78 and 79.

In view of the foregoing amendments and clarification, together with the affidavit under 37 C.F.R. § 1.132 by Dr. Wood, Applicants respectfully request withdrawal and reconsideration of the rejections under 35 U.S.C. §112, second paragraph.

Rejection Under 35 U.S.C. § 102(b) Over Noel-Hudson

Independent claims 29 and 61 and dependent claim 63, 65, and 75-79 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Noel-Hudson et al. (1993, *In Vitro Cell and Developmental Biology – Animal* 31: 508-515, reference C6 on 6/1/04 IDS). In order for a claim to be anticipated, each and every element of the claim must be present in a single prior art reference. Applicants respectfully submit that Noel-Hudson does not anticipate each of claims 29, 61, 63, 65, and 75-79, at least because Noel-Hudson does not disclose each and every element of independent claims 29 and 61, from which claims 63, 65, and 75-79 directly or indirectly depend.

Independent claims 29 and 61 recite a cell suspension comprising a composition of cells harvested from the dermis and epidermis of a skin tissue sample. The composition of cells has a cell population comprising keratinocytes basal cells, melanocytes, and fibroblasts; and the cell population of the composition and the tissue sample are comparable. Claims 29 and 61 also recite that the cell suspension is free of cellular congregates greater than 200 μM.

Noel-Hudson reports the expression and differentiation of keratinocytes cultured on cell culture inserts. The keratinocytes were isolated from human foreskin according to the methods

of Boyce, S.T.; Ham, R.G., "Cultivation, frozen storage and clonal growth of normal human epithelial keratinocytes in serum-free media." *J. Tissue Cult. Methods* 9:83-93 (1985) ("Boyce and Ham"), as reported in Noel-Hudson at page 509, column 1, paragraph 7:

> "*Cells and culture conditions*. Human keratinocytes were isolated from human foreskin of 1-yr-old donor, as described by Boyce and Ham (7). Briefly, the biopsy fragments were first treated with 0.25% trypsin (wt/vol) and 1000 U/ml collagenase (vol/vol) in Hank's solution containing Ca^{++} for 2 h at 37° C, then with a 0.025% trypsin (wt/vol): 0.01% EDTA (wt/vol) solution to release individual cells."

Drawing from this paragraph, the Office action states at pages 5-6 that "Noel-Hudson's composition lacks all cell aggregates of any size ('individual cells;" ibid.) and comprises a physiological saline, specifically Hanks' solution with calcium salts (ibid.)." The Office action points to Van Bossuyt (U.S. Pat. No. 5,866,167, "Van Bossuyt") for support that "skin (such as the skin biopsy fragments of Noel-Hudson) inherently contains keratinocytes (epithelial cells), melanocytes and Langerhans cells in the epidermis; proliferating keratinocytes at the base of the epidermis; and fibroblasts in the dermis." The Office action further states that "Van Bossuyt's teachings indicate that the whole-skin biopsy of Noel-Hudson inherently contains all of the cell types recited in claims 29, 61 and 65." From this, the Office action concludes that "[t]he cited art taken as a whole demonstrates a reasonable probability that the suspension of the prior art is either identical or sufficiently similar to the claimed suspension that whatever differences exist are not patentably significant." According to the Office action, "[c]lear evidence that the suspension of the cited prior art does not possess a critical characteristic that is possessed by the claimed suspension (e.g., the presence of all of the recited cell types) would advance prosecution and might permit allowance of claims to applicant's suspension."

In response, Applicants respectfully submit that the cell suspension reported at page 509, column 1, paragraph 7 of Noel-Hudson is neither identical nor similar to the cell suspension claimed in the instant invention. Noel-Hudson states that the keratinocytes were isolated using the method described by Boyce and Ham. Turning to Boyce and Ham, each step used in the isolation and primary culture of human keratinocytes is described starting at page 85, Section H. In brief, at step 5, Boyce and Ham describe exposing 4-mm fragments of human foreskin to

collagenase under certain conditions until the "epidermis is readily removable from dermis." (Emphasis added.) At step 7, Boyce and Ham provide "[f]or each piece of tissue, remove the epidermis from the dermis by holding the dermis with fine mouse-tooth forceps and pulling the epidermis from the edge with needle-tipped forceps. The epidermis should separate as an intact sheet." (Emphasis added.) At step 8, Boyce and Ham provide "[a]s each piece of tissue is separated, place the epidermal layer in a 60-mm petri dish containing Solution A at room temperature. The dermis is discarded, unless it is used to culture dermal cell types."[2] (Emphasis added.) Boyce and Ham go on at step 9 to provide that the epidermal sheets are treated as follows: "[A]dd 6 ml of a solution of 0.025% trypsin (wt/vol) and 0.01% EDTA (wt/vol) in Solution A (pH 7.4). Gently agitate the epidermal fragments up and down in a cotton-plugged, sterile Pasteur pipette for 3 to 4 min to release individual cells…Using a sterile Pasteur pipette, withdraw the cell suspension from the remaining tissue fragments…and centrifuge..." (Emphasis added.) At step 10, Boyce and Ham provide that the pellet from centrifugation is resuspended "by gentle pipetting until it is broken into ***many small clumps***. Release more individual cells form the epidermal pieces in the petri dish by repeating the agitation…Transfer the resulting cell suspension (but not the remaining tissue fragments) from the dish…" (Emphasis added.) Boyce and Ham next describe centrifuging the cell suspension, and then at step 11 provide as follows: "[a]spirate the trypsin-inhibitor or serum solution from the pellet, resuspend the pellet in 2 to 3 ml of supplemented stock culture medium and gently pipette until the pellet is dispersed into a suspension of single cells ***(with some small clumps remaining)***. Count the cells…and inoculate the cells into pre-equilibrated flasks…" (Emphasis added). Boyce and Ham then provide for culturing, subculturing, and freezing. No further filtering or cell separation is described in Boyce and Ham.

Taken together with Boyce and Ham, Noel-Hudson reports at page 509, column 1, paragraph 7, a cell suspension comprising individual cells, along with small clumps, derived from the epidermis of a whole skin tissue sample. Nothing in Noel-Hudson teaches or suggests a

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[2] Boyce and Ham show in FIG. 1 that the dermis and epidermis are separated from each other and that only epidermis is subject to further treatment to release individual cells. Further, Applicants respectfully note that Noel-Hudson discloses the isolation of keratinocytes from the epidermis, and does not the use of the dermis to culture for dermal cells. Indeed, Noel-Hudson discloses "[c]ell suspensions of keratinocytes … in the absence of fibroblasts." Noel-Hudson at Abstract, emphasis added.

cell suspension free of cellular congregates greater than 200 μM. To the contrary, the cell suspension of Noel-Hudson includes small clumps—the brief description of isolating keratinocytes and the reference to releasing individual cells in Noel-Hudson corresponds to step 9 of Boyce and Ham which, at steps explicitly describes small clumps of cells remaining in the cell suspension. Furthermore, Noel-Hudson fails to teach or suggest, inherently or otherwise, a cell suspension that has a cell composition having a cell population comprising keratinocyte basal cells, melanocytes, ***and fibroblasts***—i.e., a composition of cells harvested from both the epidermis (e.g., keratinocyte basal cells and melanocytes) ***and the dermis*** (e.g., fibroblasts)[3]. Moreover, Noel-Hudson completely fails to teach or suggest a suspension having a cell composition that is comparable in cell population with the tissue sample from which the composition was produced—a tissue sample comprising both dermis and epidermis. In fact, because Noel-Hudson teaches separating the epidermal layer from the dermal layer and using only the epidermal layer of the skin tissue sample to produce the cell suspension, Noel-Hudson necessarily teaches away from the cell suspension of claims 29 and 61.

In addition to the foregoing, the affidavit under 37 C.F.R. § 1.132 by Dr. Wood further emphasizes that Noel-Hudson's composition is neither identical nor substantially similar to Applicants' cell suspension. See, for example, paragraph 5B where Dr. Wood states that: "Boyce removes the epidermis from the dermis, discards the dermis, uses a Pasteur pipette to agitate the epidermal fragments, and releases keratinocytes from the epidermal pieces. Boyce at pages 85-88. At least because the epidermis is separated from the dermis before any cell suspension is prepared, and because the cell suspension prepared by gentle pipetting contains many small clumps (thus not free of cellular congregates greater than 200 μM), Noel-Hudson's composition (in view of Boyce) is different from U.S.S.N. 10/068,299. That is, at no time does Noel-Hudson produce a composition that is either identical or substantially similar to the cell suspension of U.S.S.N. 10/068,299."

For at least the foregoing reasons, Applicants submit that claims 29 and 61 are patentable over Noel-Hudson. Claims 63, 65, and 75-79 are dependent upon claim 61, and thus are also patentable over Noel-Hudson. Accordingly, Applicants respectfully request that the rejection of

[3] Applicants respectfully refer the Examiner to Van Bossuyt at column 1, lines 40-50, which reports that keratinocytes and melanocytes are located in the epidermis, while fibroblasts are located in the dermis.

claims 29, 61, 63, 65, and 75-79 under 35 U.S.C. § 102(b) over Noel-Hudson be reconsidered and withdrawn.

Rejection Under 35 U.S.C. § 102(b) Over Hirobe

Independent claims 29 and 61 and dependent claim 63, 65, and 75-79 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hirobe (1991, *Journal of Experimental Zoology* 257: 184-194; reference U; "Hirobe 1991"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Hirobe 1991 does not anticipate each of claims 29, 61, 63, 65, and 75-79, at least because Hirobe does not disclose each and every element of claims 29 and 61, from which claims 63, 65, and 75-79 directly or indirectly depend.

Independent claims 29 and 61 recite a cell suspension comprising a composition of cells harvested from the dermis and epidermis of a skin tissue sample. The composition of cells has a cell population comprising keratinocytes basal cells, melanocytes, and fibroblasts; and the cell population of the composition and the tissue sample are comparable. Claims 29 and 61 also recite that the cell suspension is free of cellular congregates greater than 200 μM.

According to the Office action at pages 8 and 9, Hirobe 1991 teaches "a composition comprising cells dissociated form mouse whole skin tissue by cutting the tissue into small pieces and incubating the pieces tissue (sic) in a 0.25% solution of trypsin (page 185 under "Culture of melanocytes"). The composition of Hirobe is a suspension of single cells (*Id.*). and comprises a serum-free physiological saline…" The Office action further provides that "[i]n this case, this rejection might be overcome by a substantive evidentiary showing that the method steps recited in the cited claims produce a composition that is materially and patentably distinct from the skin cell suspension of Hirobe….The discussion of inherent properties in the rejection over Noel-Hudson also applies to this rejection for similar reasons." In addition, the Office action states that "[t]he composition of Hirobe is produced from a sample that has been enzymatically dissociated; therefore, the composition contains those cells that were present in the tissue sample. There is no disclosure in Hirobe that any cells have been destroyed, so the amount of each type

of cell relative to each other is comparable to that in the tissue; "comparable" is not synonymous with "identical"."

At page 185, column 1, under "Culture of melanocytes," Hirobe 1991 reports that skin tissue samples taken from mice were "cut into small pieces (5 X 5 mm^2) and incubated in 0.25% trypsin…in phosphate-buffered saline (PBS, pH 7.2) for 14-16 hours at 2° C. *Epidermal sheets were mechanically separated from the dermis with fine forceps* and floated onto 0.02% solution of ethylenediamine-tetra-acetate (EDTA…) in CMF-PBS in plastic centrifuge tubes." (Emphases added.) Hirobe 1991 goes on to report that the tubes were gently shaken and incubated at 37° C for 15 minutes. Afterwards, "the tissues were gently shaken repeatedly to produce a suspension of basal cells, and the cornified sheets were removed. Then the suspension of *epidermal cells* was gently and repeatedly introduced into and expelled from a Pasteur pipette to generate a suspension of single cells." (Emphasis added.) The cells were then pelleted by centrifugation and resuspended.

While Hirobe 1991 reports a suspension that includes single cells, nowhere does Hirobe 1991 teach or suggest, explicitly or inherently, that such suspension was free of all cellular congregates greater than 200 μM. Hirobe 1991 does not teach or suggest that the epidermal tissue or cells were subjected to any filtration or cell separation techniques beyond "gently" shaking, and "gently and repeatedly" pipetting the suspension in CMF-PBS with EDTA. One skilled in the art would readily appreciate that the method described by Hirobe 1991 would not inherently produce a suspension comprising only single cells in the absence of any cellular congregates greater than 200 μM. Furthermore, Hirobe 1991 completely fails to teach or suggest, explicitly or inherently, a cell suspension that has a composition of cells having a cell population comprising keratinocyte basal cells, melanocytes, ***and fibroblasts***—i.e., a composition cells harvested from both the epidermis (i.e., keratinocyte basal cells and melanocytes) ***and the dermis*** (i.e., fibroblasts). As such, Hirobe 1991 necessarily fails to teach or suggest a suspension having a cell composition that has a cell population that is comparable with the cell population with the tissue sample comprising dermis, epidermis and a dermal-epidermal junction therebetween. In fact, because Hirobe 1991 reports separating the epidermis from the dermis and producing a "suspension of epidermal cells" from the skin tissue sample,

Hirobe 1991 teaches away from the cell suspension of claims 29 and 61. As such Hirobe 1991 fails to teach each and every element of the instant claims.

In addition to the foregoing, the affidavit under 37 C.F.R. § 1.132 by Dr. Wood further emphasizes that Hirobe 1991's composition is neither identical nor substantially similar to Applicants' cell suspension. See, for example, paragraph 5C where Dr. Wood states that: "Hirobe at page 185 describes mechanically separating epidermal sheets from the dermis, preparing epidermal cells from the epidermal sheets, and gently and repeatedly pipetting the epidermal cells to produce a cell suspension. At least because the epidermis is separated from the dermis before any cell suspension is prepared, and because the cell suspension prepared by gentle pipetting is not free of cellular congregates greater than 200 μM, Hirobe's composition is different from U.S.S.N. 10/068,299. That is, at no time does Hirobe produce a composition that is either identical or substantially similar to the cell suspension of U.S.S.N. 10/068,299."

For at least the foregoing reasons, claims 29 and 61 are patentable over Hirobe 1991. Claims 63, 65, and 75-79 are dependent upon claim 61, and thus are also patentable over Hirobe 1991. Accordingly, Applicants respectfully request that the rejection of claims 29, 61, 63, 65, and 75-79 under 35 U.S.C. § 102(b) over Hirobe 1991 be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that the claims, as amended, are in condition for allowance and request favorable action. The Examiner is invited to contact Applicants' Attorney at the number below if in the Examiner's view it would expedite the prosecution of the application.

Respectfully submitted,

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